B. C. PATIL*:

Cytology and nature of ploidy in three species of *Crotalaria*

B. C. パティル*: タヌキマメ属3種の倍数体

The tetraploid species of *Crotalaria* reported so far are only eight, although the genus is distributed in tropical and warm temperate regions of both hemispheres with about 550 species. Majority of the tetraploids are confined to warm temperate regions only.

It is of interest to note that there exists uniformity in diploid chromosome number (2n=16) in about 90% of reported species (Chennaveeraiah & Patil 1972, Nadakarni 1979). Eight tetraploid species reported are *C. nitens*, *C. paulina*, *C. stipularia*, *C. pumila*, *C. pilosa*, *C. foliosa*, *C. velutina* and *C. manaiensis* (Boulter *et al.* 1970, Chennaveeraiah & Patil 1972, Nadakarni 1979, Coleman & De Menezes 1980).

Cytological studies on tetraploids is very meager. The present investigation has been taken up with two-fold purpose, that is, primarily to study the karyotype and secondarily to explore the nature of ploidy.

Material and methods The source of material for the present study is given below:

1)	*Crotalaria paulina* Schrank.	Brazil
2)	*C. stipularia* Desv.	Sweden
3)	*C. nitens* H. B. K.	Sweden

The mitotic study was made from healthy root tips following Tjio & Levan's (1950) oxyquinoline aceto-orcein squash method. Meiotic preparations were made by fixing suitable flower buds in Carnoy's fixative for 2-3 h. Slides were stained with aceto-carmine.

The chromosome morphology reveals that they are of medium size. The length variation in a complement is not much. Therefore, the chromosomes are classified on the basis of position of centromere and presence or absence of satellites. The chromosome types recognized are:

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Type S″: Chromosomes with submedian centromere with interstitial satellites.
Type V : Chromosomes with median centromere.
Type J : Chromosomes with submedian centromere.

Observations

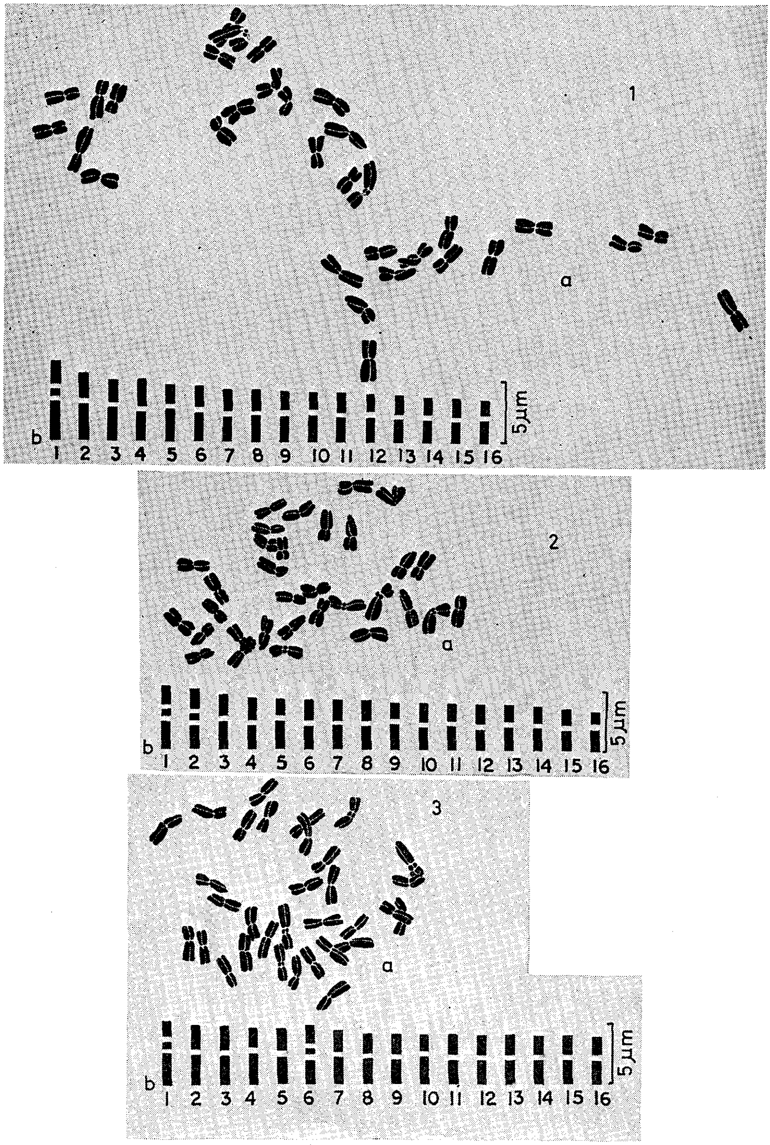
1) *C. paulina* Schrank. 2n=32=2S″+8V+22J. The karyotype of this species appears as asymmetrical one, with varying in length between 2.57 and 4.53 μm, with an absolute length of 51.95 μm (Figs. 1a, b & 13). The chromosome set contains three types of chromosomes. The first pair is submedian chromosome with interstitial satellites, the satellite measuring 0.41 μm and is the longest pair (Fig. 1b). The second type of chromosomes are with median centromeres consisting of four pairs. All other chromosomes are with submedian centromeres and form the third type.

This species did not flower in our experimental garden. Therefore, meiotic studies could not be carried out.

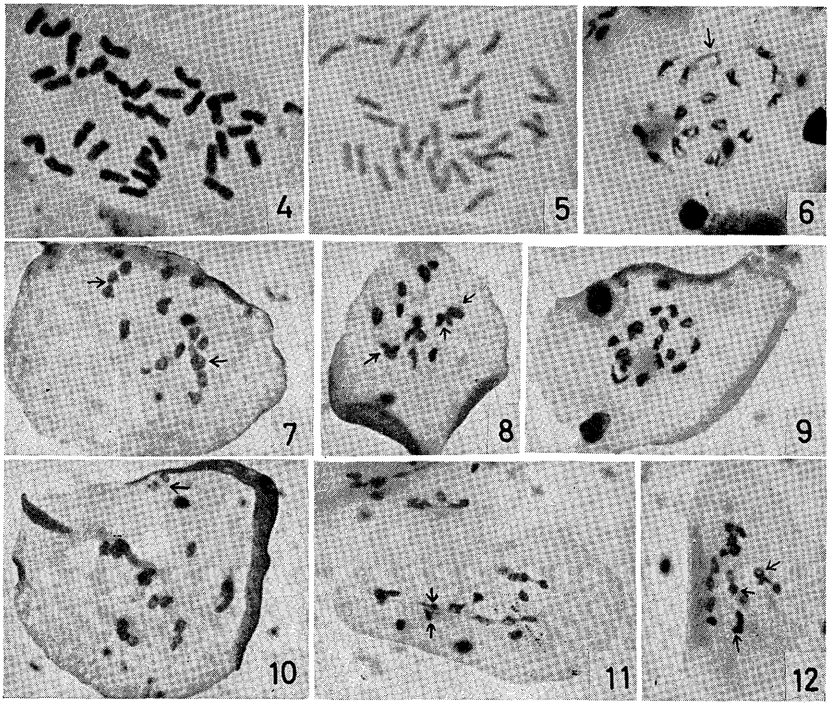
2) *C. stipularia* Desv. 2n=32=4S″+4V+24J. There are three types of chromosomes in the karyotype (Figs. 2a, b & 4). The first type has two pairs of submedian chromosomes with interstitial satellites, the satellites measuring 0.41 μm, in both the pairs. These two pairs occupy the first and second position in the idiogram (Fig. 2b). There are two pairs of chromosomes with median centromeres forming the second type. The remaining twelve pairs of chromosomes with submedian centromeres represent the third type. The chromosome length varies from 2.30 to 3.59 μm, with an absolute length of 46.77 μm.

Meiotic studies in this species reveals that, although sixteen bivalents are formed at metaphase-I, in some cells quadrivalents are encountered at diakinesis and M-I (Figs. 6, 7 & 8). The frequency of quadrivalents at M-I ranges from 1-3, with an average of 0.253 per cell (Tab. 2). About 86.67% of the PMCs show complete bivalent formation. Irregularities as recorded in Tab. 3 are seen during various stages of meiosis. Except for these irregularities meiosis is normal.

3) *C. nitens* H. B. K. 2n=32=4S″+2V+26J. The karyotype shows similarity with that of Chennaveeraiah & Patil's report (1973). There are three types of chromosomes (Figs. 3a, b & 5). First pair being submedian chromosome with interstitial satellites is the longest pair, another pair of such chromo-



Figs. 1-3. Karyotypes (a) and idiograms (b) of *Crotalaria*. 1. *C. paulina*, 2. *C. stipularia*, 3. *C. nitens*.



Figs. 4–5. Chromosomes in metaphase, ×1100. 4. *C. stipularia*, 5. *C. nitens*. Figs. 6–12. Chromosomes in meiosis, ×550. 6–8. *C. stipularia*. 6. Diakinensis: 14II+1IV. 7. Metaphase-I: 12II+2IV. 8. Metaphase-I: 10II+3IV. 9–12. *C. nitens*. 9. Diakinensis: 16II. 10. Metaphase-I: 2I+9II+3IV. 11. Metaphase-I: 5II+2III+4IV. 12. Metaphase-I: 10II+3IV. Arrow indicates univalents in Fig. 10, trivalents in Fig. 11, and quadrivalents in Figs. 6–8 and 12.

some occupies the sixth position in the idiogram (Fig. 3b). The satellites measure 0.41 μm in both the pairs. There are two pairs of chromosomes with median centromeres forming the second type. The rest of thirteen pairs of chromosomes have submedian centromeres and represent the third category. The longest chromosome in complement measures 3.78 μm and the shortest 2.82 μm, with an absolute length of 51.11 μm.

Meiotic observations show that, there are sixteen bivalents in about 97.21% of the cells at metaphase-I (Tab. 2). However, quadrivalents, trivalents, and univalents are met in about 2.71% of the cells. The frequency of quadrivalents, trivalents and univalents at M-I ranged from 3-4, 0-2, and 0-2 with an average of 0.089, 0.016 and 0.007 per cell respectively (Figs. 10, 11 & 12) (Tab. 2). Meiosis is normal except for the irregularities as shown in Tab. 3.

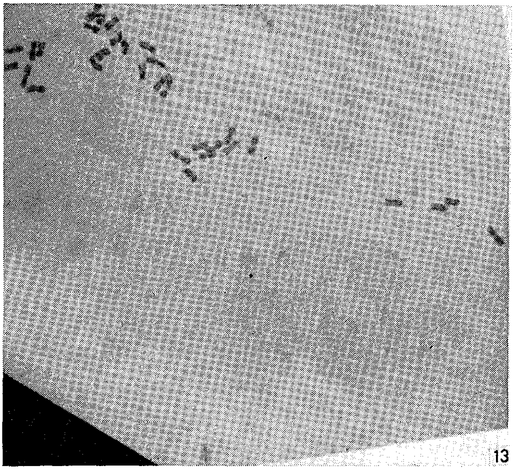


Fig. 13. Somatic metaphase of *C. paulina* 2n=32, ×61.

Discussion Chromosome number for *C. paulina* has been reported as 2n=32 by Shibata (1962), and as 2n=16 by Datta & Mondal (1968). The present study shows that it is 2n=32. Turner & Irwin (1961) have reported the chromosome number as n=16 for *C. stipularia.* Present report of chromosome number is 2n=32 and n=16.

In the karyotypes of these taxa, there are more number of submedian chromosomes (Tab. 1) indicating that these species are in intermediary position in course of evolution in the genus *Crotalaria.* In comparison among three tetraploids the karyotype of *C. nitens* is more advanced, because of high number of submedian chromosomes (Tab. 1).

The chromosome markers such as interstitial satellited submedian chromosomes of tetraploid and diploid taxa reveal that there are distinct two pairs in tetraploids (*C. stipularia* and *C. nitens*) and one pair in diploids (Chennaveeraiah & Patil 1972). In diploids these occupy first position in the idiograms as observed in thirty-three diploid species (Patil 1970). The comparison between these chromosome markers of the tetraploids and corresponding pair of diploids show that they are morphologically resemble each other but their length and arm ratios vary. In case of *C. paulina* there is only one pair of interstitial satellited submedian chromosome, which is the longest pair in the idiogram (Fig. 1b). Whereas in *C. stipularia* and *C. nitens* there are two pairs of such chromosomes. The position of these chromosomes in the idiogram of these species is first, second and first, sixth in *C. stipularia* and *C. nitens* respectively

Tab. 1. Comparison of somatic chromosomes in three tetraploid species of *Crotalaria*.

Species	2n No.	No. of different types of chromosomes			No. of interstitial sats.	Range of chromosome length in μm	Absolute length in μm
		S″	V	J			
C. paulina	32	2	8	22	2	2.57–4.53	51.95
C. stipularia	32	4	4	24	4	2.30–3.59	46.77
C. nitens	32	4	2	26	4	2.82–3.78	51.11

Tab. 2. Metaphase-I chromosome configuration in *C. stipularia* and *C. nitens* 2n=4X=32.

Species	I	II	III	IV	No. of cells	% of total cells
C. stipularia	—	14	—	1	08	5.33
〃	—	12	—	2	06	4.00
〃	—	10	—	3	06	4.00
〃	—	16	—	—	130	86.67
Average	—	15.50	—	0.253	Total: 150	100.00
Total no. of chromosomes	—	4648	—	152	4800	

Species	I	II	III	IV	No. of cells	% of total cells
C. nitens	2	9	—	3	01	0.40
〃	—	5	2	4	02	0.71
〃	—	10	—	3	04	1.60
〃	—	16	—	—	250	97.21
Average	0.007	15.793	0.016	0.089	Total: 257	99.92
Total no. of chromosomes	2	8118	12	92	8224	

Tab. 3. Meiotic observations in *C. stipularia* and *C. nitens*.

Stages	Irregularities	*C. stipularia* No. of cells	*C. stipularia* No. of normal cells	*C. stipularia* % of irregular cells	*C. nitens* No. of cells	*C. nitens* No. of normal cells	*C. nitens* % of irregular cells
Anaphase-I	Laggards	12	521	2.30	04	669	0.60
〃	Unequal distribution	01	194	0.51	03	426	0.70
〃	Irregular distribution	01	050	2.00	01	298	0.34
〃	Precocious movement of chromosomes	08	476	1.68	01	138	0.68
Telophase-I	Micronuclei	01	194	0.51	02	390	0.52
〃	〃	01	050	2.00	02	050	0.40

(Figs. 2b & 3b). Such changes in length and morphology of chromosomes may be due to reciprocal translocations as observed in segmental allotetraploids (Schulz-Schaeffer *et al.* 1963). Similarly on the basis of chromosome morphology the situation of *C. paulina*, *C. stipularia* and *C. nitens* 4X may be a case of segmental allotetraploid.

The meiotic metaphase-I pairing configuration frequencies as shown in Tab. 2 if, compared with the expected frequencies of autotetraploids that is one-third bivalents and two-third quadrivalents as suggested in a model by Jackson & Casey (1980) or two to three quadrivalents per cell as reported by Dewey (1979); then the total number of chromosomes in bivalents should be 1600 (33.3%) and 2741 (33.3%) but 4648 (96.8%) and 8118 (98.7%) were observed in *C. stipularia* and *C. nitens* respectively. Total chromosomes in quadrivalents should have been 3200 (66.6%) and 5483 (66.6%) but only 152 (3.2%) and 92 (1.11%) or 0.253, 0.089 quadrivalents per cell were observed in *C. stipularia* and *C. nitens* respectively. Thus the cytological data of these tetraploids is in non-agreement with those of required for an autotetraploid, but strongly indicates that at least tetraploids *C. stipularia* and *C. nitens* are derived through segmental allotetraploidy.

Although the various kinds of irregularities are seen at meiosis, their percentage is very low (Tab. 3). Which does not effect the pollen fertility in both the species.

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マメ科タヌキマメ属の 3 種 *Crotalaria paulina*, *C. stipularia*, *C. nitens* は 4 倍体, 2n=32 であり，それぞれの核型は本文中に示した。この倍数体は異質倍数体と考えられる。